Pulpal blood flow recorded from exposed dentine with a laser Doppler flow meter using red or infrared light

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ABSTRACT

Objective: To determine the percentage of the blood flow signal that is derived from dental pulp when recording from exposed dentine in a human premolar.

Design: Recordings were made from 7 healthy teeth in 5 subjects (aged 22-33 yr.) with a laser Doppler flow meter (Periflux 4001) using either a red (635 nm) or an infrared (780 nm) laser. After exposing dentine above the buccal pulpal horn (cavity diam. 1.6 mm, depth 3 mm) and isolating the crown with opaque rubber dam, blood flow was recorded alternately with infrared or red light from the exposed dentine under four conditions: before and after injecting local anaesthetic (3% Mepivacaine without vasoconstrictor) (LA) over the apex of the root of the tooth; after exposing the pulp by cutting a buccal, class V cavity in the tooth; and after sectioning the coronal pulp transversely through the exposure.

Results: There was no significant change in mean blood flow recorded with either light source when the tooth was anaesthetized or when the pulp was exposed. After the pulp had been sectioned, the blood flow recorded with infrared light fell by 67.8% and with red light, by 68.4%. The difference between these effects was not significant.

Conclusions: When recording blood flow from exposed coronal dentine with either infrared or red light in a tooth isolated with opaque rubber dam, about 68% to the signal was contributed by the pulp. The signal:noise ratio was better with infrared than red light, and when recording from dentine than enamel.

1. Introduction

Laser Doppler flow meters have been used to record blood flow from dental pulp for thirty years, since the original studies of Gazelius, Olgart, Edwall & Edwall (1986) and Olgart, Gazelius & Lindh-Strömberg (1988). This technique has the principal advantage over alternative methods that it is non-invasive. Also, continuous recordings can be obtained. Disadvantages are that the records cannot be calibrated in absolute units (Vongsavan & Matthews, 1993), and records from the crown of a tooth always include a component that is derived from tissues outside the tooth, such as the gingiva and periodontal ligament, as well as the pulp (Soo-ampon, Vongsavan, Soo-ampon, Chuckpaiwong & Matthews, 2003).

Most of the observation in humans have been made by placing the laser Doppler probe on enamel in the bucco-cervical region of a tooth. Under these conditions, the contamination of the records from non-pulpal tissues can be reduced by covering the adjacent gingiva with opaque dam or some other suitable material. For example, Soo-ampon et al. (2003) found that the use of opaque rubber dam reduced the blood flow signal recorded from an intact, anterior tooth in man by an average of 73%. Akpinar, Er, Polat & Polat (2004) used an opaque periodontal paste to cover the gingiva with similar results. Soo-ampon et al. (2003) also showed that the signal remaining after applying opaque rubber dam was further reduced by 57% when the pulp was removed and replaced in the root canal. Thus only approximately 10% of the signal recorded from a tooth without dam, and 43% of that recorded with dam could be attributed to blood flow in the pulp.

Laser Doppler blood flow records can be obtained with different wavelengths of light, which do not all penetrate tissues to the same extent. For example, infrared light (780 nm) penetrates deeper than red light (635 nm) (Bonner & Nossal, 1990), thus a record from the cervical region of a tooth that was obtained with infrared light might be expected to include a greater contribution from tissues outside the pulp than one obtained with red light. However, Kijsamanmith, Timpawat, Vongsavan & Matthews, (2011a, 2011b) found no such difference. With both wavelengths, and using opaque rubber dam, the proportion of the signal due to dental pulp was around 46 % for anterior teeth and 60 % for premolars.

Another limitation often encountered when trying to record pulpal blood flow through enamel with a laser-Doppler flow meter is that the signal obtained is very weak and close to the limit of resolution of the instrument (Soo-ampon et al., 2003). Banthitkhunanon, Chintakanan, Wanachantararak, Vongsavan & Matthews (2013) showed that it might be possible to avoid this problem by recording from exposed dentine. Blood flow signals obtained from exposed dentine in deep cavities were more than 10X those obtained from the corresponding area of enamel. This result was obtained in an *in vitro* study in which diluted blood was pumped through the pulp cavity. More recently, Sukapattee, Wanachantararak, Sirimaharaj, Vongsavan & Matthews (2016) found that pulpal blood flow values recorded from exposed dentine in premolars and molars during and after cutting minimum depth, full-crown preparations were about double those recorded from the corresponding area of enamel prior to the crown preparation

The aim of the present experiments was to determine what proportion of the blood flow signal recorded from dentine near the tip of a cusp of a premolar was derived from the pulp. Both red (635 nm) and infrared (780 nm) light were tested to determine if, in this location, the wavelength of the light would affect the result.

2 Materials and Methods

The experiments were carried out on 7 healthy premolar teeth in 5 human subjects (aged 22-33 yr., mean 27.3). These teeth were scheduled to be extracted for orthodontic purposes. All the teeth were intact. Radiographic examination and electrical pulp stimulation confirmed that they were vital and healthy.

The experiments were carried out in the Endodontics Clinic of the Maha Chakri Sirithon Dental Hospital at the Salaya Campus of the Faculty of Dentistry, Mahidol University. The study was approved by the Ethics Committee of Faculty of Dentistry/Faculty of Pharmacy, Mahidol University, Institutional Review Board (Certificate no. COA.No.MU.DT/PY-IRB 2015/049.0610) and complied with the principles of the Declaration of Helsinki. The experimental procedures were clearly explained to each subject and their written consent to these procedures being carried out was obtained. The subject could terminate the experiment at any stage. The privacy rights of the subjects were observed at all times.

2.1 Cavity preparation

A 1.6 mm diameter, 3.0 mm deep cavity directed towards the pulp horn was cut at the tip of the buccal cusp. The enamel was first removed with a 1.0 mm diam. round, diamond bur in an air-rotor drill under a constant stream of water. The cavity was then deepen and the floor flattened with a 1.6 mm diameter, tungsten carbide, flat fissure bur in a very slow hand-piece and with copious water spray. The enamel surface around the cavity was etched with 35% phosphoric acid (3M ESPE; St. Pual, MN, USA) and a cannula made from a 4 mm length of 16G stainless steel needle (ext. diam. 1.59 mm, int. diam. 1.19 mm) was sealed into the cavity with flowable composite resin (3M ESPE; St. Pual, MN, USA), care being taken to avoid the resin flowing onto the cavity floor.

2.2 Pulpal blood flow recording

Blood flow recordings were made with a Periflux System 4001 two-channel, laser Doppler flow meter (Perimed AB, Järfälla, Sweden). One channel was equipped with an infrared (780 nm) laser and the other, a red (635 nm) laser. Time constants of 0.03 s were used for the infra-red channel, which showed any rapid fluctuations in blood flow; and 0.2 s for the red, which showed slower changes with a better signal:noise ratio. Measurements of mean blood flow over several seconds were not affected by these settings. The blood flow data were recorded from the digital output of the flow meter with a computer running the PeriSoft (version 1.13) software program.

The flow meter probe (type 415-159) had an ext. diam. of 1.0 mm and contained 2 optical fibres (diam. 0.125 mm, separation 0.25 mm). The probe was inserted into the cannula in the cavity until it touched the exposed dentine (Fig. 1). Light was transmitted between the dentine and the probe through air; no attempt was made to improve the optical contact between them by introducing an alternative contact medium. Recordings were made with the probe connected alternately to each of the two channels of the flowmeter. The probe was zeroed separately with infrared and red light and calibrated according the manufacturer’s instructions so that the Brownian motion of a standard suspension of latex particles (Vongsavan & Matthews, 1993) gave a reading of 250 arbitrary perfusion units (P.U.).

Fig. 1. near here

After the cavity at the tip of the tooth had been prepared and the cannula inserted, the crown was isolated by the application of opaque black rubber dam (Four D Rubber Co. Ltd., Heanor, England). Blood flow recordings were made under four conditions: (1) before and (2) after the tooth had been anaesthetised by a submucosal injection of local anaesthetic (1.7 ml Mepivacaine 3% without vaso-constrictor, Septodont; Saint-Maur-des-Fossés, France) administered buccally over the apex of the test tooth (3) after exposing the pulp by cutting a buccal, class V cavity in the tooth; and (4) after sectioning the coronal pulp transversely with the tip of a fine scalpel blade (No.11) through the exposure. ~~At each stage, the blood flow signal was allowed to stabilize for several minutes~~ After each intervention, the blood flow signal was allowed to stabilize before further measurements were made. The time required for this, and in the case of the local anaesthetic injection also for the anaesthetic to take effect, was typically between 3 and 5 minutes. ~~before then~~ Measurements were made from a recording of approximately 15 seconds duration. The total backscattered light intensity was also noted.

After each experiment, recordings were also made at different light intensities from a stationary reflector (white card). These data were used to calculate the offset of the blood flow signal that would have been present due to noise in the detection system while recording from the teeth (Vongsavan & Matthews, 1993). For each blood flow recording obtained during an experiment, the mean and standard deviation values of blood flow were calculated in P.U. and the offset appropriate for the intensity of backscattered light present, determined as described above, was subtracted from the mean.

After the experiments, the tooth was extracted with forceps and sectioned bucco-lingually through the cusps with a diamond disc and water coolant. The remaining dentine thickness was measured between the floor of the tip cavity and the closest point of the pulp chamber.

2.3 Statistical Analysis

Comparisons between the overall mean blood flow values recorded under each of the different conditions were made using one–way, repeated measures analysis of variance (ANOVA). Where this showed that there were significant differences between the means, the Tukey test was used to make multiple comparisons between them. The mean blood flow values that were obtained under corresponding conditions with the two light sources were compared with Student’s paired t-test. The statistical analyses were carried out with Sigmaplot® software (version 12, Systat Software Inc., CA, USA).

P values of less than 0.05 were considered significant.

3. **Results**

A full set of data was obtained with both infrared and red light from every tooth. Examples of the records obtained from one tooth under the different conditions of the experiment are shown in Fig. 2.

With infrared light, ANOVA indicated that there was a significant difference between the mean blood flow values recorded under different conditions. After cutting the tip cavity and application of rubber dam, the mean value was 7.05 (S.D. 3.77, n=7) P.U. The administration of the local anaesthetic resulted in a fall in the mean to 5.31 (S.D. 3.16) P.U. but this change was not significant. After cavity preparation to expose the pulp, the mean value (5.68 , S.D. 3.67 P.U.) also did not change significantly. After sectioning the coronal pulp however, the mean blood flow signal decreased significantly ($P<0.05$, Tukey test) by 67.8% to 1.63 (S.D. 1.30) P.U.

A similar result was obtained with red light. The mean after preparing the tip cavity and applying the rubber dam was 3.44 (S.D.2.13, n=7) P.U. This fell insignificantly to 3.12 (S.D. 1.60) P.U. after the administration of local anaesthetic. Cavity preparation to expose the pulp produced no significant change in the mean blood flow value (3.26, S.D. 2.84P.U.) whereas sectioning the coronal pulp significantly($P<0.05$, Tukey test) reduced this value by 68.4% to 0.94 (S.D. 0.77) P.U.

A summary of the data is shown in Fig.3. The mean blood flow values obtained with infrared light were higher than the corresponding values for red light under all the experimental conditions. The differences between the mean values obtained with infrared and red light after cutting the tip cavity and application of rubber dam, after the administration of local anaesthetic, and after cavity preparation to expose the pulp were all significant (P = 0.008, 0.018, and 0.007 respectively, *Student's paired t-test*); but the difference between the means after sectioning the pulp was not significant.

The mean % reduction in the blood flow signal following sectioning of the pulp (67.8% with infrared and 68.4% with red light), provides an estimate of the contribution of pulpal blood flow to the signal obtained before this was done. The difference between these values was not significant (P=0.920, *Student's paired t-test*).

Figs. 2 & 3 near here.

The infra-red records did not reveal any high frequency fluctuations in blood-flow such as a dicrotic notch in the pulsations synchronised to the heart-beat.

The average remaining dentine thickness under the tip cavity was 1.87 (S.D. 0.19) mm.

4. **Discussion**

This study confirmed the earlier finding (Banthitkhunanon et al., 2013) that a much larger laser-Doppler blood flow signal can be recorded from exposed dentine than from enamel. Thus, by recording from dentine, the method does not have the disadvantage often encountered when recording from enamel in human teeth, that the signal amplitude is close to the limit of resolution of the flow meter (Soo-ampon et al., 2003). This would apply particularly in elderly individuals since pulpal blood flow tends to decrease with age (Ikawa, Komatsu, Ikawa, Mayanagi & Shimauchi, 2003). Another method of improving the signal:noise ratio in recordings of pulpal blood flow would be to reduce the upper limit of the bandwidth of the system that measures the Doppler shift in frequency of the light when it is scattered by moving blood cells, from the usual 24 kHz or 20 kHz (as in the present experiments) to 5 kHz (Qu, Ikawa & Shimauchi, 2014). This optimises the detection system to match the relatively slow peak blood velocities in a tooth.

It was also demonstrated that when recording from exposed dentine at the tip of the cusp of a premolar, with the crown isolated with opaque rubber dam, approximately 70% of the signal was derived from pulp as opposed to tissues outside the tooth . This is higher than has been found in studies in which recordings were made from enamel in human teeth, where the proportion was between 43 and 60% (Kijsamanmith et al., 2011a, 2011b; Soo-ampon et al., 2003). Kijsamanmith, Timpawat, Vongsavan & Matthews (2011b) recorded from the cervical buccal enamel of premolars using methods that were otherwise very similar to those of the present study and found that 60% of the signal was from pulp. Although the proportion of the signal derived from pulp was increased by recording from dentine, there was still a considerable contribution from other tissues. When recording from the enamel of deciduous teeth in pigs, Vongsavan & Matthews (1996) demonstrated that up to 85-93% of the signal was from pulp. Although these teeth are of similar size to human teeth, the difference between the results of that and the present study may be due to differences in the size of the pulp, which is larger (Vongsavan & Matthews, 1996), and in the thickness of the enamel and dentine which is smaller, in pig incisors . Although the recordings were made from exposed dentine in the present study, on average 1.9 mm remained over the pulp.

Isolating the crown of a tooth with opaque rubber dam is known to decrease the contribution of tissues outside the pulp to the blood flow signal recorded. Without black rubber dam, only approximately 10% of the signal recorded from human enamel was from pulp (Soo-ampon et al., 2003). Polat, Er, Akpinar & Polat (2004) also found that the contribution was between 3 and 14% when recording without covering the gingiva. The effect of dam may be to compress the gingival margin and thus reduce its blood flow (Ikawa, M., Personal Communication), as well as to screen the tissues from the light of the flow meter. The dams in common use clinically are not as opaque as the material used in the present study. The use of an opaque dam, or some other similar procedure to cover the gingiva, is therefore essential to ensure that the majority of the laser Doppler signal recorded from the crown of a tooth is derived from the pulp.

The proportion of the signal recorded with dam that was derived from the pulp in the present study was the same for both red and infrared light, as found in a previous study under similar conditions but recording from the buccal enamel surface of premolars (Kijsamanmith et al., 2011b). It therefore appears that the greater penetration of tissues by infrared light (Bonner & Nossal, 1990) results in a deeper penetration into the pulp as well as the surrounding structures. The signals obtained from the pulp with infrared were consistently larger than those with red light.

The performance of a laser Doppler flow meter in recording from dentine would be expected to be improved by introducing a suitable fluid with a high refractive fluid as a coupling medium between the laser Doppler probe and exposed dentine surface. This interface was occupied by air in the present experiments. Preliminary experiments carried out while recording both blood flow and nerve action potentials (Wanachantararak, Ajcharanukul, Vongsavan & Matthews, 2016) from exposed dentine at the tip of a cusp of a human premolar indicate that liquid paraffin may be a suitable medium for this purpose.

The higher resolution of the records from dentine compared with those from enamel, did not reveal any additional details of fluctuations in blood flow synchronised to the heart- beat, such as a dicrotic notch, which might have been present due to the very low compliance of the pulp (Matthews & Andrew, 1995). In recordings from enamel, Qu et al. (2014) found that blood flow in the pulpal circulation was characterized by a lack of high velocity components.

It is surprising that exposure of the pulp did not produce an increase in its blood flow. This may have been because the local anaesthetic blocked a neurogenic inflammatory response.

In conclusion, when recording pulpal blood flow with a laser Doppler flow meter a larger signal is obtained, and a greater proportion of that signal is derived from pulp, when the probe is placed on exposed dentine rather than enamel. When recording from dentine, infrared light gives a larger signal than red light but the proportion of that signal that is derived from the pulp is unaffected. Dentine may become exposed and accessible for recording as a result of attrition, trauma or cavity preparation. The method could be particularly useful for making repeated measurements of blood flow from a tooth to monitor changes in the severity of pulpitis, the effects of medication, or the progress of pulpal revascularization.

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Figure Legends

Fig. 1. Diagram of the experimental set up (not to scale).

Fig. 2. Laser Doppler records of blood flow recorded with infrared (A) and red (B) light from exposed dentine in one tooth after the preparation of the tip cavity and applying opaque black rubber dam (Baseline), after the administration of the local anaesthetic (LA), after exposing the pulp through a cervical cavity (Pulp exposed), and after sectioning the pulp (Pulp cut). The final record in each case is a control obtained with the same intensity of back-scattered light from a stationary reflector (White card). For the records obtained with infrared light, a time-constant of 0.03 s was used; whereas for red light, it was 0.2 s.

Fig. 3. Mean values of the pulpal blood flow signal recorded from 7 teeth with infrared (black columns) and red (grey columns) light after the preparation of the tip cavity and applying opaque black rubber dam (Baseline), after the administration of the local anaesthetic (LA), after exposing the pulp through a cervical cavity (Pulp exposed), and after sectioning the pulp (Pulp cut). In each case, the value was corrected by subtraction of the reading from the white card. The error bars indicate one S.D.

Figures

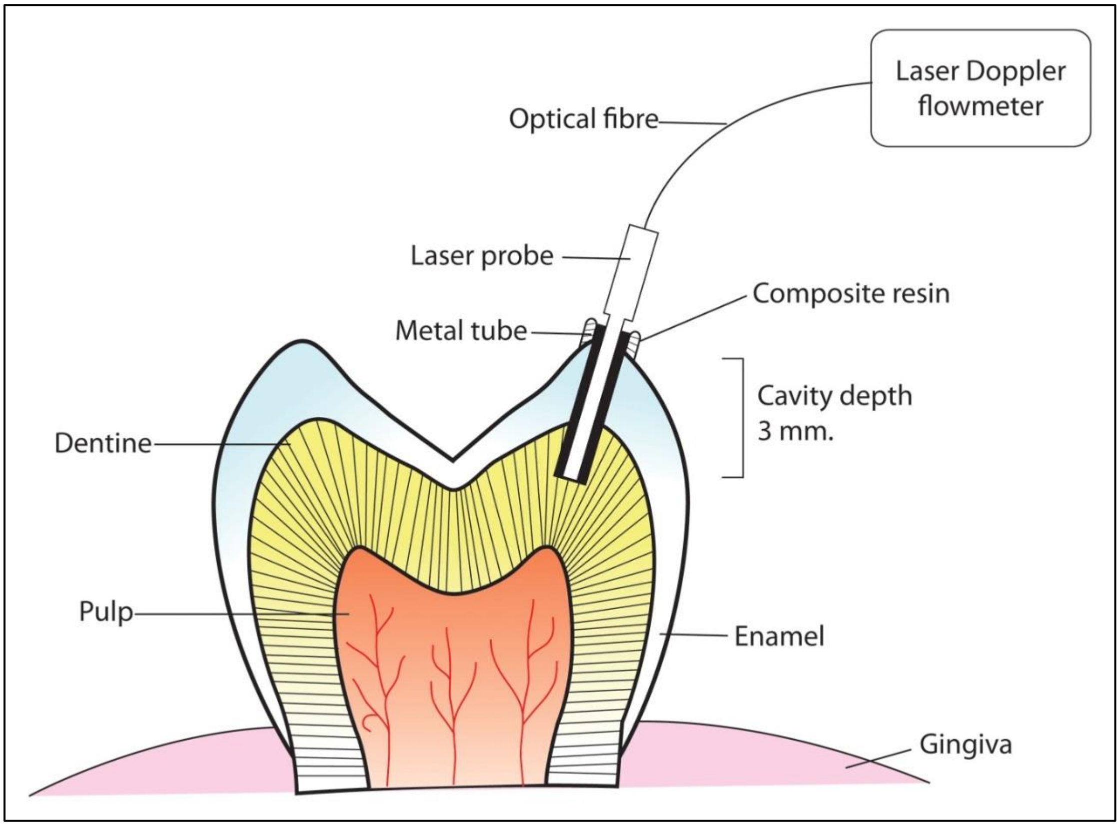
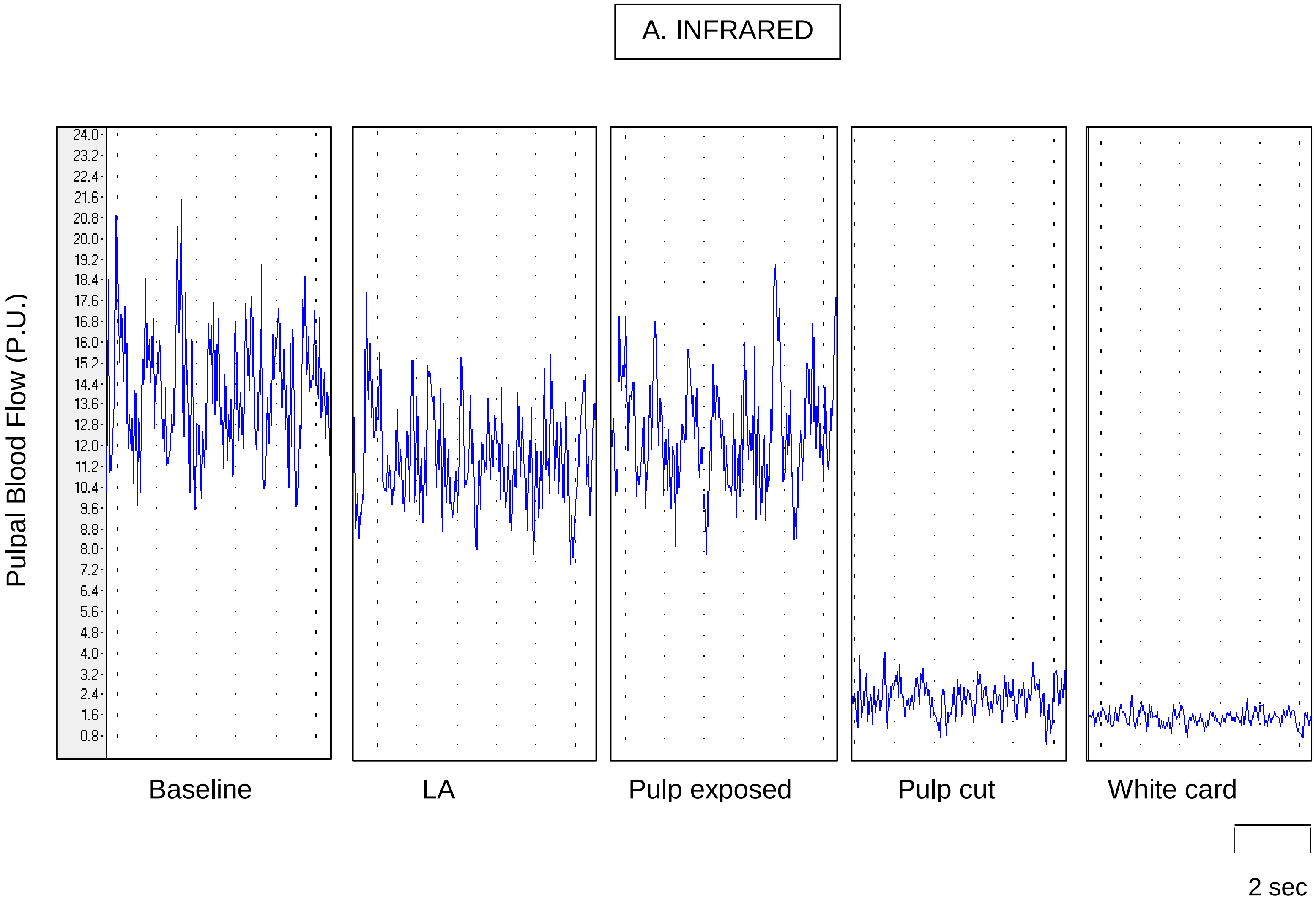


Figure 1


A. INFRARED
Pulpal Blood Flow (P.U.)
24.0
23.2
22.4
21.6
20.8
20.0
19.2
18.4
17.6
16.8
16.0
15.2
14.4
13.6
12.8
12.0
11.2
10.4
9.6
8.8
8.0
7.2
6.4
5.6
4.8
4.0
3.2
2.4
1.6
0.8
Baseline
LA
Pulp exposed
Pulp cut
White card
2 sec

B. RED

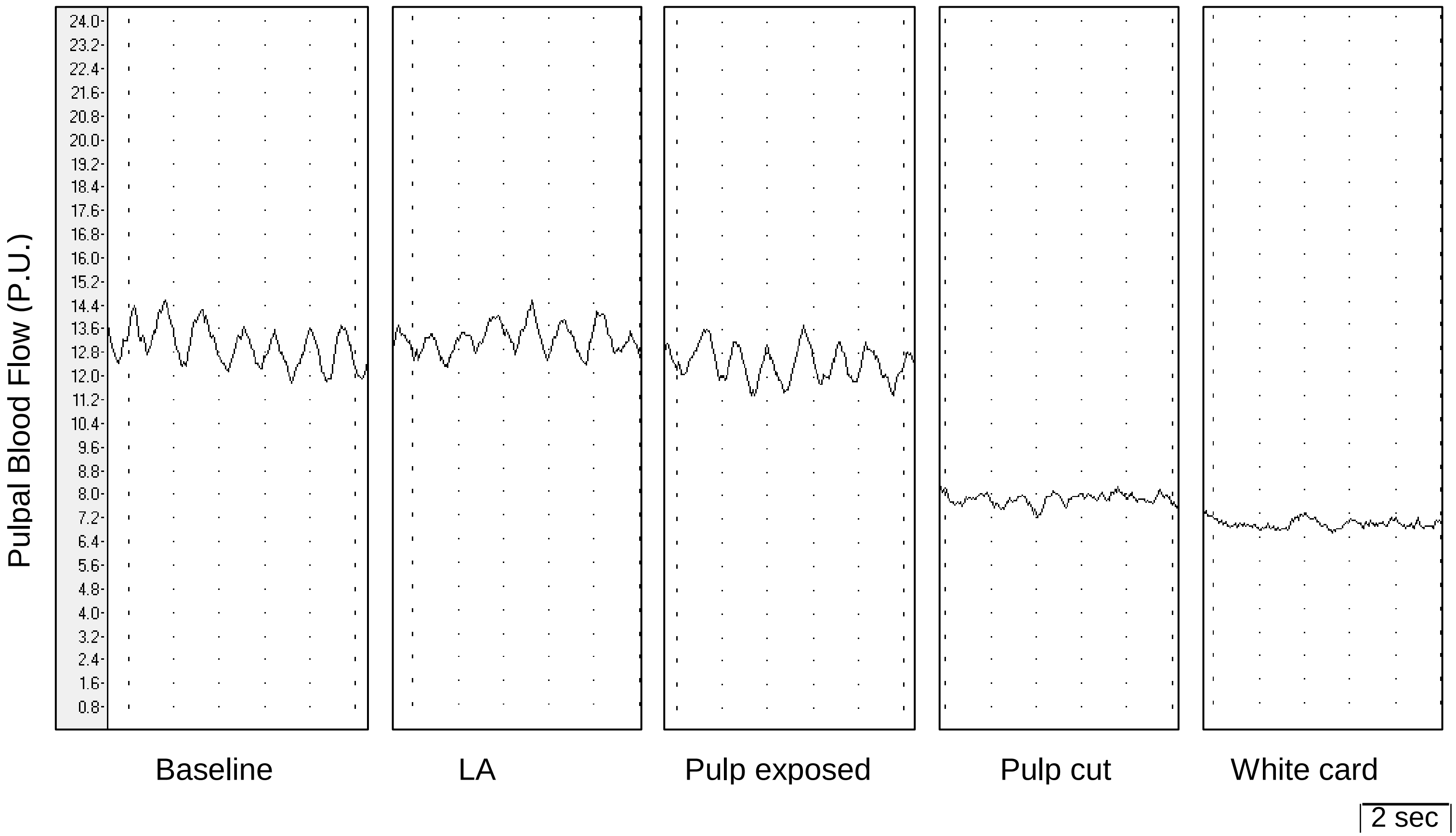


Figure 2

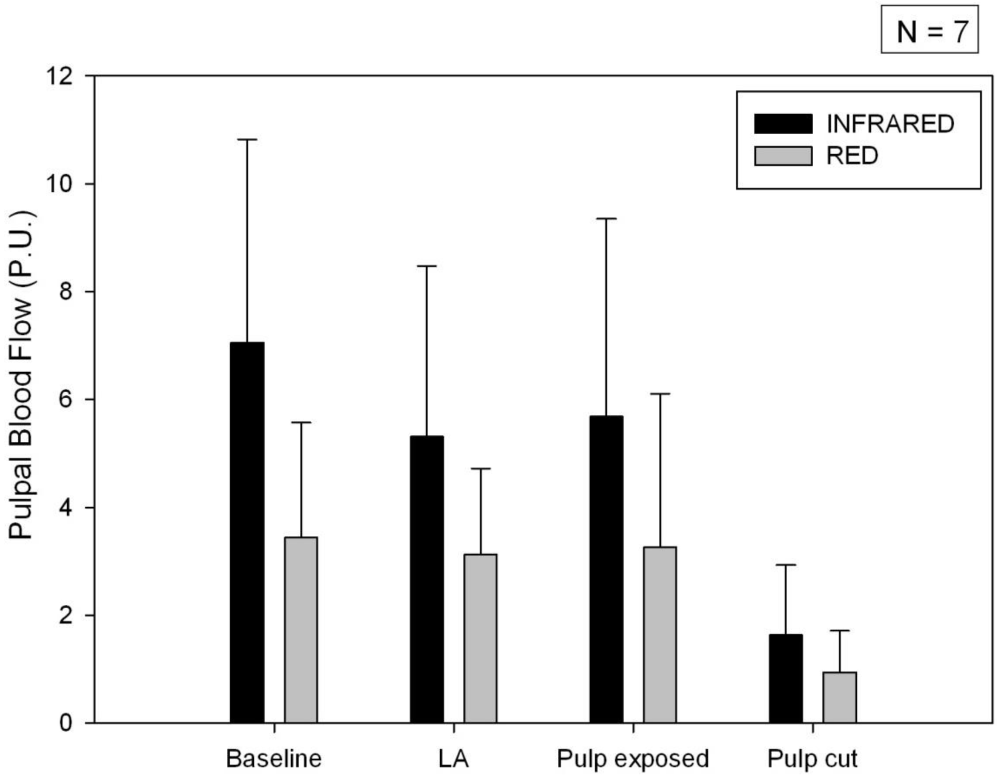

N = 7
INFRARED
RED
Pulpal Blood Flow (P.U.)
0
2
4
6
8
10
12
Baseline
LA
Pulp exposed
Pulp cut

Figure 3